Profile of Roy H. Doi

War is a defining act; it can both shape and destroy nations, and it leaves lasting legacies for the individuals touched by it. Roy H. Doi is one such individual. If it wasn't for war, he may not have ended up where he is today, a Distinguished Professor of Molecular Biology at the University of California, Davis (Davis, CA) and a member of the National Academy of Sciences (elected in 2006).

Doi is a leader in advancing understanding of the mechanisms of RNA polymerase, sigma factors, ribosomes, transfer RNA, and other components of the machinery that control bacterial gene expression. Since 1989, he has been interested in a second type of bacterial machinery, the cellulosome. This complex of enzymes and anchoring proteins is secreted by several bacteria so they can break down cellulose and other components of the plant cell wall into sugars. In his Inaugural Article (1) in a recent issue of PNAS, he and his colleagues demonstrate that different bacterial strains can express individual components of the cellulosome and still assemble a functional unit.

It may seem odd that someone who has spent more than 4 decades studying the regulation of gene activity in the bacterium *Bacillus subtilis*, a rather unassuming career choice, would be involved in global conflicts, but in his youth Doi had two experiences that defined his professional aspirations. First, as a son of Japanese immigrants to the United States, Doi spent several years in Japanese-American internment camps during World War II, an experience that developed his perseverance in the face of adversity. Second, he served in the U.S. Army in Japan on the heels of the Korean War, where he was first exposed to, and decided to pursue, scientific research.

Doi at his home in Davis, CA.

Humble and Hostile Times

Doi was born in Sacramento, CA, in 1933 and grew up in the nearby farming town of Loomis. His father came to the United States from Japan in 1903 and, like many immigrants in the American West, worked as a migrant laborer, struggling to support his family. As if these circumstances were not trying enough, in 1942 Doi and his family were forced by the U.S. government to relocate to an internment camp. He remembers that his father sold the family car for $50 because they were only allowed to take minimal possessions with them.

"We spent the next 3 years in camps at Tule Lake, California, and Heart Mountain, Wyoming," he says. "Fortunately, my parents were quite philosophic; their Asian culture had helped them develop a sort of fatalism, and they accepted what happened to them." Their outlook influenced Doi, and, like his parents, he says he is not bitter over his internment.

Conditions did not improve much once Doi and his family were released at the end of the war, however. Doi began high school in Auburn, CA, and admits that many people were still hostile toward the Japanese. "So I spent a lot of time alone in the library," he says. "I think my first year I basically lived there and probably read about 125 books." One of his favorite books was *Microbe Hunters* by Paul de Kruif (2), which planted the seeds of science in Doi's mind.

After completing high school, Doi went to Placer Junior College, also in Auburn. He was unsure what career he wanted to pursue, but he was determined to further his education. "Japanese immigrants like my father were not formally educated," he says, "but they saw all the successful people in the community—doctors, dentists, lawyers—and [encouraged] their kids to become educated. But since they didn't go to college, they couldn't give me too much advice." Doi started out in pre-med, and after 2 years he transferred to the University of California, Berkeley (Berkeley, CA), where he completed a degree in physiology in 1953. With the United States in the midst of the Korean War, however, Doi did not have time to celebrate; as soon as he graduated, his military deferment was over and he was drafted into the U.S. Army.

The Virus Hunter

Upon completing military basic training, Specialist Roy Doi was sent to Camp Kilmer, NJ, where he was stationed with a genito-urethral research project (GURP) ward. "They had this ward because many GIs were coming back from Korea with venereal disease, and the colonel in charge there was interested in finding the cause for these outbreaks," says Doi. He remembers that the colonel thought that the disease outbreaks might have been psychosomatic, as a means for the soldiers to get discharged.

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"I became curious about this, and when I talked to the colonel, who was an M.D., about whether these were psychosomatic versus bacterial or viral, it really piqued my interest in microbial research."

That interest intensified when Doi was sent to Japan and assigned to a medical unit studying Japanese encephalitis (similar to West Nile disease). He was placed on the mosquito search team, tasked with finding virus-harboring mosquitoes so the researchers could study their life cycle. "I was pretty naïve," he says, "so I asked the person in charge, 'How do you become a researcher?' He told me I had to go to graduate school, and I responded, 'What's that?' I had heard of medical school or dental school, but I had never heard of graduate school."

To prepare for graduate school, Doi returned to Berkeley when he was discharged from the army in 1955, and he completed a second undergraduate degree in bacteriology. One of Doi's professors, Clint Ballou, suggested that Doi try the University of Wisconsin (Madison, WI) for graduate school. "That's where [Ballou] received his Ph.D., and he told me that they had a very good program in microbiology and biochemistry." Following Ballou's advice, Doi went to Wisconsin and studied bacterial sporulation under Harlyn Halvorson. "Harlyn was a very supportive advisor," says Doi. "In fact, the entire faculty was very supportive. And growing up in a Japanese-American community in California—my Boy Scout group was all Japanese, my church was all Japanese—it was good to get away. The type of people I met and the ideas I received at Wisconsin really broadened my horizons."

Studying Spores

After completing his dissertation on the metabolism of bacterial spores in 1960 (3), Doi took a postdoctoral position at the University of Illinois at Urbana–Champaign (Urbana, IL) with microbiologist Sol Spiegelman, who was interested in the replication of RNA viruses. "I think Sol was the only real genius I have been associated with," says Doi. "And his complete devotion to science really impressed me. When you're having Thanksgiving dinner and your boss calls you up and asks, 'How did your experiment go?' you know he's serious."

Doi arrived in Wisconsin at an opportune time to learn about molecular biology. Between Watson and Crick cracking the genetic code in 1953 and Jacob and Monod explaining how the *lac* gene could be turned on and off 8 years later, Doi got to witness the birth of the gene-expression era. He remembers with particular fondness the Cold Spring Harbor meeting of 1961 (Cold Spring Harbor, NY) that he attended with Spiegelman, where Doi stood shoulder to shoulder with the world's top molecular biologists.

During his postdoc years, Doi had remained interested in unicellular morphogenesis, so when he started his own laboratory at Syracuse University (Syracuse, NY) in 1963, he switched back to studying bacterial sporulation, using *B. subtilis* as the model. However, he made sure he brought some of Spiegelman's devotion with him. "I kept my nose to the grindstone," he recalls. "I would typically work 7 days a week; I sort of resented weekends." He also used his newfound molecular biology techniques. "I learned about DNA/RNA hybridization from Spiegelman, and in one of my first experiments, I used that to show that new families of messenger RNA were being made during sporulation, which demonstrated that differential gene expression was occurring" (4).

"When you're having Thanksgiving dinner and your boss calls . . . you know he's serious."

Doi returned to the familiar surroundings of Sacramento in 1965 after he was offered a position at the University of California, Davis. The change in scenery did not slow his prodigious output, however, and over the next 2 decades he continued exploring the molecular biology and genetics of *Bacillus*. Among his notable works was his finding that sporulating Bacilli contain multiple forms of RNA polymerase (5). This finding led to his discovery that multiple sigma factors—small proteins that guide RNA polymerase to specific gene promoters—direct this enzyme, not just one. Doi also happily notes that he showed that the ribosomal RNA sequence of different *Bacillus* species was conserved (6). This finding took prominence a few years later, after Carl Woese and George Fox used 16S ribosomal RNA conservation as their basis for establishing Archaea as a separate kingdom of life (7).

Breaking Down Barriers

In 1989, Doi was appointed chair of a committee tasked with assessing the biotechnology programs at the University of California, Davis. Having spent more than 25 years working on basic questions about bacterial gene expression, he was quite a novice in this area. As he learned about biotechnology applications, he heard that the California State Legislature was going to pass a law that would outlaw rice straw burning by 2001. "Every year after the rice harvest, the farmers burned the leftover stalks," says Doi. "And this black smoke would go into the air and ash would spread into the city, creating a lot of pollution." Although this legislation was good for the environment, it posed a quandary regarding the vast amount of straw that had to be destroyed. "And I thought to myself, instead of burning it, why don't we degrade it with enzymes?"

So, Doi switched the primary focus of his work to the breakdown of cellulose. He chose the recently discovered sporulating bacteria *Clostridium cellulovorans* as his model because it produces copious amounts of extracellular cellulolytic enzymes that come together in a large complex. Then, after a crash course in anaerobic microbiology, he got to work, trying to uncover the structure and roles of all of the subunits of the complex.

He initially hypothesized that the largest subunit, which was noncatalytic, served as the core protein that coordinates the activity of the remaining catalytic subunits (8). He deduced the sequence of this 190-kDa subunit and found that it indeed has nine enzyme binding sites (9). Over the next decade, Doi was largely responsible for building a comprehensive picture of this scaffolding protein, called CbpA, and of the multitude of cellulases that dock with it, together known as the cellulosome (10). He humbly adds, "I received tremendous support from Paul Stumpf at U.C. Davis and Arnold Demain at MIT, among others."

Recently, Doi demonstrated that *C. cellulovorans* genes can be expressed in *B. subtilis* and that the proteins will still be secreted into the medium (11). That finding led him to wonder whether expressing genes in multiple bacteria would also produce a functional cellulosome, a question he tackles in his Inaugural Article (1). Doi found that if two different *B. subtilis* strains, one expressing the CbpA docking protein and the other an enzyme component, are cocultured, the components come together in the medium and become functional; this cooperation worked for both glucanase and xylanase enzymes. "There are other cases of bacteria cooperating with each other, such as quorum sensing," says Doi, "but I'm not aware of any instance of an active enzyme being formed by

two different bacteria." Unsure of what to call this remarkable phenomenon, Doi discussed it with his Davis colleague John Roth, who suggested "intercellular complementation."

The Davis Project?

Although the scientists and engineers will undoubtedly do their part and improve cellulose breakdown, there may simply not be enough arable land to sustain a growing world population. "I have this crazy idea, though," says Doi, "that we could use the ocean as a farm to grow and harvest marine plants to use as a source of fuel." Doi has not been able to sell others on this idea yet, but as the discussion of alternative fuels progresses, he may find more supporters. With the recent commercial implementation of ethanol-fueled vehicles, biofuels created from degraded plant biomass have become a large area of interest. and researchers such as Doi have been trying to maximize their applicability. "Right now they're using corn, but even if they used all the current resources, stalks and all, they would only meet 30% of our energy needs," he says. "And you know, that land and that fertilizer could probably be better used to grow food."

Regardless of the source, however, a top priority in making ethanol fuel commercially viable is improving the efficiency of the breakdown of cellulose. Doi notes, "Right now, the process of producing ethanol from plants is about 10 times more costly than oil, so we have to reduce that to be competitive." Although these facts seem daunting, Doi thinks it is only a matter of time until the problem is solved. "All the ideas are already there; we just have to make it better. I have great confidence in American molecular biologists and engineers; if you give them a problem, they can solve it."

To speed up this process, Doi thinks the United States may want to learn from a defining moment when war and science mixed: the Manhattan Project to develop the atomic bomb during World War II. If the world's top scientists and engineers in biofuel research were to be locked in a room together today, in short order they might be able to harness the power of the cellulosome the same way they conquered the atom decades earlier.

Nick Zagorski, *Science Writer*

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